DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA MOLECULE PROGRAM ADVISORY COMMITTEE

MINUTES OF MEETING

SEPTEMBER 13-14, 1976

The Recombinant DNA Molecule Program Advisory Committee was convened for its sixth meeting at 9:00 a.m. on September 13, 1976 in Wilson Hall, Building 1, National Institutes of Health, Bethesda, Maryland. Dr. DeWitt Stetten, Jr., (Chairman) Deputy Director for Science, and Dr. Leon Jacobs, (Vice Chairman) Associate Director for Collaborative Research, NIH, presided. In accordance with Public Law 92-463, on September 13, from 9 a.m. to 5 p.m., and on September 14, from 9 a.m. to 3 p.m., the meeting was open to the public. The meeting was closed to the public on September 14 from 3 p.m. to 5 p.m for the review, discussion and evaluation of initial, pending, and renewal grant applications containing detailed research protocols, designs, and other technical information.

Committee members present were:

Drs. Edward A. Adelberg; Roy Curtiss, III; James E. Darnell, Jr.; Peter Day; Donald R. Helinski; Elizabeth M. Kutter; Emmette S. Redford; Wallace P. Rowe; Jane K. Setlow; John Spizizen; Waclaw Szybalski; LeRoy Walters; and William J. Gartland, Jr., Executive Secretary.

A Committee roster is attached. (Attachment I)

The following ad hoc consultants to the Committee were present:

Dr. David Botstein, Massachusetts Institute of Technology, Cambridge, MA
Dr. Allan Campbell, Stanford University, Stanford, CA
Dr. Rolf Freter, University of Michigan Medical School, Ann Arbor, MI
Dr. Susan Gottesman, National Cancer Institute, NIH
Dr. Margaret Lieb, University of Southern California, Los Angeles, CA
Dr. Ann Skalka, Roche Institute of Molecular Biology, Nutley, NJ
Dr. Francis Ruddle, Yale University, New Haven, CT

The following liaison representatives were present:

Dr. Richard Hedrich, National Endowment for the Humanities
Dr. Herman Lewis, National Science Foundation
Dr. Elena Nightingale, National Academy of Sciences

Other National Institutes of Health staff present were:

Mr. Manuel Barbeito, NCI; Dr. Emmett Barkley, NCI; Dr. Fred Bergmann, NIGMS; Mrs. Betty Butler, NIGMS; Dr. Irving Delappe, NIAID; Dr. Lynn Enquist, NICHD; Dr. Michael Goldberg, NIGMS; Dr. Robert Goldberg, NCI; Mrs. Florence Hassell, NIGMS; Mr. Joe Hernandez, OD; Dr. John Irwin, DRS; Dr. Elke Jordan, NIGMS; Dr. Ruth Kirschstein, NIGMS; Dr. Malcolm Martin, NIAID; Dr. John Nutter, NIAID; Dr. Joseph Perpich, OD; Dr. Warren Powell, DRS; Dr. Maxine Singer, NCI; Dr. Bernard Talbot, OD; Dr. David Tiemeier, NICHD, and Dr. Katherine Wilson, DRG.

Others in attendance for all or part of the meeting were:

Dr. Frederick Blattner, University of Wisconsin; Dr. J. R. DeZeeuw, Central Research, Pfizer, Inc.; Dr. Rosa Gryder, Food and Drug Administration; Dr. James McCullough, Congressional Research Services, Library of Congress; Mr. Colin Norman, Nature; Dr. Oliver Smithies, University of Wisconsin; Dr. Charles Weiner, Massachusetts Institute of Technology; Dr. Michael Yarmolinsky, FCRC.

I. CALL TO ORDER

Dr. Stetten called the meeting to order and introduced Dr. LeRoy Walters, who was attending his first meeting as a committee member. Dr. Stetten announced that the meeting was being tape-recorded. He mentioned that the committee members had been sent a questionnaire by Dr. Robert F. Rushmer, a member of the Advisory Panel on Decision-Making on R&D Policies and Priorities in the Office of Technology Assessment. Committee members are to respond directly to Dr. Rushmer. Dr. Stetten said that the NIH is preparing an analysis of the Draft Report To The Canadian Medical Research Council from its Ad Hoc Committee on Guidelines for Handling Recombinant DNA Molecules and Certain Animal Viruses and Cells. He asked committee members to send comments directly to Dr. L. Siminovitch, Chairman of the Canadian Ad Hoc Committee on Guidelines, with a copy of the correspondence to Dr. Leon Jacobs.

II. CONSIDERATION OF MINUTES

The Minutes of the April 1-2, 1976 meeting were considered. Two members said that they felt that the Minutes were not prepared in sufficient detail. Subject to the comments the Minutes were accepted as written.

III. PROPOSED HIGH CONTAINMENT (P4) FACILITY AT FREDERICK CANCER RESEARCH CENTER

Dr. John Seal, Deputy Director, NIAID, made a presentation on a possible regional or national P4 facility at the Frederick Cancer Research Center (FCRC). NIAID has assumed responsibility for providing P4 containment in cooperation with NCI and NIGMS. With the aid of a diagram, Dr. Seal described the second floor of Building 567 at FCRC, which was previously operated at a high containment level. The area in question comprises 9200 square feet of laboratory space. Conversion of the first floor of Building 567 to high containment could also be considered. This would greatly increase the space available for animal holding. Renovation of

the second floor would cost $600,000 to $1 million, and would take 6-12 months. The facility would require a director and a minimum of 10 to 12 people as core staff. It would be able to handle 3 or 4 investigators with 1 or 2 technicians each. The steady state operating costs of the facility would be approximately $300,000 to $500,000 per year. Dr. Seal stressed that there needs to be assessment of the potential use of such a facility, taking into consideration whether investigators are willing to leave their laboratories and move to a central P4 facility, and what equipment, layout and technical skills are required. Some of these questions will be asked in the next issue of NARSM. Details for the review of applications for use of the facility would have to be developed. Proposals could possibly be reviewed by the NIH Biohazards Committee, the Office of Recombinant DNA Activities, and/or the Recombinant DNA Molecule Program Advisory Committee. At present it is contemplated that such a facility would be used only for recombinant DNA experiments. Dr. Yarmolinsky stated that FCRC has a recombinant DNA committee and that FCRC staff should be consulted regarding establishment and operation of a P4 facility.

In response to a question, Dr. Barkley reviewed other potential P4 facilities. He stated that there are approximately 30 facilities with the potential for P4 containment. Of these, approximately 5 or 6 could possibly be certified now as P4 facilities. He pointed out that most of these facilities are already dedicated for other uses. An NIH committee has recommended that the NIH should proceed with only one P4 facility at the present time until the level of potential utilization is more accurately determined. It was pointed out that the need for P4 containment may diminish in the future due to the availability of EK3 host-vector systems and techniques for <u>in vitro</u> replication of DNA.

Following this discussion, the Committee unanimously passed a resolution stating that a national P4 facility should be established by the NIAID.

IV. <u>NATIONAL RECOMBINANT DNA FACILITY</u>

Dr. Francis Ruddle, Chairman of the NIGMS Mammalian Cell Lines Committee, presented a proposal for the establishment of a national recombinant DNA facility (Attachment II). The Committee noted that this is a separate concept from the provision of a generally available P4 containment facility, as discussed above. The emphasis here is on a repository of cloned DNA segments. The repository may require P4 containment at the outset, but in the future it might be able to operate at a lower containment level. It was felt that the quality of this facility will depend on its director, and that its setting should be in a good scientific environment. Dr. Kirschstein indicated that the NIGMS would be willing to take the responsibility for exploring the feasibility of such a facility. There was discussion as to whether the facility should bank DNA segments other than human. Dr. Ruddle said that the concept focuses on human DNA, although mouse and viral DNA segments would also be of interest. An advisory committee to the facility would have to set priorities.

The Committee unanimously passed a motion that it supports the concept of investigating the establishment of a human DNA clone bank under the auspices of the NIGMS, and that an advisory committee should be appointed, which should include non-cloners and perhaps non-scientists as well as cloners, to advise on this and other clone banks.

V. BIOLOGICAL CONTAINMENT

A. Procedures for Certification of EK2 and EK3 Systems

Dr. Adelberg led a discussion on a number of issues related to EK2 and EK3 biological containment. During this discussion the Committee referred to proposals submitted by Drs. Curtiss and Freter.

The Committee unanimously recommended that the following language should be inserted into the guidelines.

"Certification of host-vector systems

Responsibility

"Certification of EK2 and EK3 host-vector systems and of the presence of a conjugative plasmid in an EK1, EK2 or EK3 host-vector system is the responsibility of the NIH Recombinant DNA Molecule Program Advisory Committee. Data on the construction, properties and testing of proposed host-vector systems will be analysed and reviewed by a subcommittee composed of one or more members of the NIH Recombinant DNA Molecule Program Advisory Committee and other individuals chosen because of their expertise in evaluating such data. Such subcommittees shall provide a written report to the NIH Recombinant DNA Molecule Program Advisory Committee. The Committee will evaluate this report and any other available information at a regular meeting. Approval of the system will require a two-thirds majority of the full Committee.

"The NIH Recombinant DNA Molecule Program Advisory Committee can rescind a certification at any time, should new data or new considerations invalidate the previous decision. In such cases, investigators may be asked to transfer their recombinant DNA into a different approved system.

"Certification of a given system does not extend to modification of either the host or vector component of that system. Such modified systems must be independently certified."

The Committee also unanimously passed a resolution that it is understood that its actions are advisory to the Director, NIH. This includes recommendations on certification of EK2 and EK3 systems.

B. Tests Required for EK2 and EK3 Certification

The Committee discussed whether in vivo testing should be required for for certification of EK2 systems.

Dr. Adelberg stated that the Working Group on Safer Hosts and Vectors, which met in June to review putative plasmid EK2 systems, using 1776 as a host, relied on rodent test data in its evaluation of EK2 properties. Dr. Adelberg felt that the guidelines should require such in vivo tests for EK2 certification. The guidelines currently require EK2 certification on the basis of suitable in vitro tests designed to represent the natural environment. Dr. Adelberg stated that EK3 would still be distinct from EK2 under this proposal, as EK3 requires primate testing. He said that he was interested in obtaining the Committee's views on the principle of requiring in vivo tests in the future for EK2 certification. The details would be elaborated by a subcommittee. The question was immediately raised as to whether putative phage EK2 systems need to be tested in vivo. There was some feeling that phage need to be considered separately.

Some members argued against a requirement for in vivo testing for EK2 certification on the basis that if this principle were adopted there would not be enough distinction between EK2 and EK3 systems, and that the concept of in vivo testing is against the spirit of EK2 systems. One Committee member stated that in vivo tests would be rapid and are in accord with previous ideas. Dr. Adelberg questioned whether totally adequate in vitro tests can be devised. Other members were concerned that there will be great disagreement about appropriate in vivo test systems. A suggestion was made that there should be an appendix to the guidelines on biological testing. The question was also raised as to who would conduct the in vivo testing if the principle is adopted. Dr. Freter, who proposed survival tests for putative EK2 hosts and vectors in rodents, said that survival in the mouse intestine is a much closer approximation to the human intestine than any tests which can be devised in vitro. He said that the proposed tests can be carried out in a matter of several days and present no great problems. It was suggested that in vivo tests might be required for the original testing of EK2 systems, while retesting could be carried out in vitro. The in vivo EK2 testing could possibly be carried out by contract. It was pointed out that the containment levels for permissible experiments were voted on the basis of EK2 systems which had been subjected only to in vitro testing requirements, rather than the presumably more stringent in vivo tests.

The Committee was then asked to vote in principle as to whether in vivo testing should be required for EK2 systems involving plasmid vectors. Six members voted in favor of the requirement for in vivo testing for EK2 systems, and six members voted against the requirement. Dr. Stetten cast a tie-breaking vote in favor of the principle of in vivo testing. A subcommittee of Drs. Adelberg, Curtiss and Szybalski was appointed to prepare proposed changes in definitions of biological containment for discussion at the next meeting.

C. Distribution of Certified Systems

The Committee discussed the centralized distribution of certified EK2 and EK3 host-vector systems and recommended the following language for insertion into the guidelines:

"Distribution of certified host-vectors

"Certified EK2 and EK3 host-vector systems will be distributed only by the Research Resources Branch, National Institute of Allergy and Infectious Diseases, NIH, or by the originator of the strain acting on NIH's behalf, following announcement by the NIH Office of Recombinant DNA Activities of the availability of the system by publication of notices in appropriate journals and NARSM. These notices will briefly describe the system and indicate the requirement that the system be obtained from the Research Resources Branch, NIAID.

"Plasmid vectors will be in a strain other than the disarmed host, and phage vectors will be distributed as small volume lysates. Investigators developing EK2 and EK3 host-vector systems will work out with the Research Resources Branch the best way of packaging cultures and phage for long-term storage and distribution. If the Research Resources Branch propagates any of the host strains or phage, a sample will be returned to the investigator developing the system or to an appropriate testing contractor for verification before distribution.

"In distributing the certified EK2 and EK3 host-vector systems, the Research Resources Branch will send out a complete description of the system, enumerate and describe the tests to be performed by the user to verify the important phenotypic traits, remind the user that any modification of the system necessitates independent certification of the system by the NIH Recombinant DNA Molecule Program Advisory Committee and remind the user of responsibility for notifying the NIH Office of Recombinant DNA Activities of any discrepancies with the reported properties or any problems in the safe use of the system."

[The Committee later recommended that changes in the guidelines proposed at this meeting be deferred for further discussion at the next meeting.]

The question was raised as to whether existing clones constructed in EK1 host-vector systems, which subsequently have been certified as EK2 systems, can be considered to be cloned in EK2 systems, or whether the clones must be transferred to certified EK2 systems obtained from the Research Resources Branch, NIAID, or the originator of the certified strain. The Committee felt that such clones can be considered to be in EK2 systems provided that the investigator had received the host-vector system directly from the originating laboratory.

D. Modification of Certified Systems

The Committee discussed procedures for handling "minor modifications" of approved EK2 systems. There was some feeling that as the full Committee is responsible for certifying EK2 and EK3 systems this responsibility should not be delegated. Other members felt that there should be some mechanism for certifying minor modification improvements of already certified EK2 systems. There was sentiment that a subcommittee should be appointed to which an investigator could communicate through the NIH as to which tests are required, so that certification of modified systems would be delayed only until the next meeting of the full Committee. The Committee passed that following motion, with 10 members in favor and none opposed:

> "Modifications of an existing EK2 vector or host should be submitted to an appropriate subcommittee or working group, which will decide what additional data are needed for certification of the modified system by the Committee."

VI. PATENTS

Dr. Perpich and Mr. Latker led a discussion on NIH patent policy regarding recombinant DNA technology as discussed in Dr. Predrickson's letter to the Committee members (Attachment III). The basic question is whether current HEW policy regarding patents is appropriate for recombinant DNA research. Mr. Latker discussed provisions of the Institutional Patent Agreement (IPA), which covers all types of inventions. He said that there needs to be sound reasons for deviation from normal HEW policies. Mr. Latker also reviewed foreign patent applications.

After discussion, the Committee members voted their preferences. Nine members recommended option No. 4, one member voted for option No. 5, and four members voted for option No. 3. None of the members voted for option No. 1 and 2.

VII. REVIEW OF PROPOSED EK2 HOST-VECTOR SYSTEMS

A. Host-plasmid systems χ1776 (pSC101) and χ1776 (pCR1)

Dr. Roy Curtiss of the University of Alabama initially submitted data on a putative EK2 host-vector system to the Committee on March 30. Because of inadequate time to consider the data, the Committee deferred consideration of Dr. Curtiss' submission at its April meeting. The data were reviewed by a Working Group on Safer Hosts and Vectors which met at the Massachusetts Institute of Technology on June 9 (Attachment IV). The Working Group concluded that E. coli strain χ1776 with plasmid pSC101, which is designated χ1776 (pSC101), meets the criteria for an EK2 system. It further concluded that χ1776 carrying plasmid pCR1, designated χ1776 (pCR1), would likely meet the criteria for an EK2 system, but that certain additional test data were required.

Dr. Adelberg summarized the Working Group report for the Committee, and called attention to crucial points in the review. Dr. Helinski submitted additional data on χ1776 (pCR1) as stipulated by the Working Group Report. Drs. Curtiss and Helinski were present in the room to answer questions from the Committee, but absented themselves during the final discussion and vote.

The question was raised as to whether the Working Group had taken into consideration the critique of the Boston Area Recombinant DNA Group on χ1776, and whether it was prepared to respond to the critique point by point. Dr. Adelberg pointed out that the comments were received in Cambridge only shortly before the Working Group met. He said that he had read the critique prior to the meeting, but that it was not discussed point by point. It was pointed out that members of the Boston Area Recombinant DNA Group were present in the room during the Working Group's meeting, and that they did not raise specific points at that meeting. Dr. Adelberg agreed to undertake a response to the Director, NIH, on the Boston Area critique.

Eight members of the Committee voted that χ1776 (pSC101) and χ1776 (pCR1) meet the criteria for EK2 host-vector systems. There were two abstentions. No members voted against the motion.

Dr. Curtiss was asked to publish information on the properties and use of this strain in NARSM, and perhaps elsewhere.

B. Host-phage systems

1. Report of Working Group on Safer Hosts and Vectors

Dr. Szybalski introduced a report from the Working Group on Safer Hosts and Vectors (Phage Systems) which had met on September 12. Dr. Gottesman presented a discussion of the possible modes of escape of the phage, or of a permissive host carrying the phage. The Working Group proposed certain standardized laboratory tests that would be required for EK2 certification (Part I of Attachment V). Dr. Adelberg stated that the subcommittee on definitions of biological containment will seriously consider inclusion of the tests proposed by the Working Group. The report of the subcommittee will be considered at the next meeting of the full Committee.

During the review of the Working Group's report, the question of the desirability of requiring in vivo tests of phage systems was discussed. Concern was raised about the ingestion of lambda phage. Some members of the Working Group and the Committee felt that in vivo tests, while relevant for plasmid systems, are not relevant for phage systems. Other members felt that most of the in vitro tests for phage systems are better, but that some in vivo testing, if easy, would be desirable to test certain properties.

2. Charon host-phage systems

The Committee reviewed the recommendations of the Working Group regarding data on Charon vector systems submitted by Dr. Frederick Blattner, et al. of the University of Wisconsin (included in Part II of Attachment V). Dr. Gottesman, who had voted against the certification of these systems in June, said that she now had no reservations concerning the recommendations of this Working Group report regarding the Charon systems. She pointed out that data on these systems had been intensively reviewed by Working Groups for a total of 13 hours.

The Committee voted on a motion to accept the recommendations of the Working Group on the Charon systems (Page 7 of Attachment V). Eleven members voted in favor of the motion. There was one absention. No negative votes were cast.

3. λWES.λB host-phage system

Dr. Szybalski presented the recommendations of the Working Group on the λWES.λB system submitted by Dr. Philip Leder, et al. of the NIH. The Committee reviewed the Working Group's recommendations and voted on a motion to accept them (Page 8 and 9 of Attachment V). Ten members voted in favor of this motion. There was one absention. No negative votes were cast.

The question was raised as to the status of the λgt Wam403 Eam1100 Sam100.λC vector of Leder, Enquist and Tiemeier which had been certified as EK2 by the Committee at its April meeting. There was general agreement that the λB vector reviewed by the Committee at this meeting is safer than the λC system recommended by the Committee at its last meeting. Dr. Leder said that he would send the λB vector to everyone who had received λC. There was some sentiment that the λC vector should be "retired" sometime in the future.

VIII. BIOHAZARD ASSESSMENT EXPERIMENTS

Dr. Rowe reported on the current status of biohazard assessment experiments involving polyoma virus, being conducted in collaboration with Dr. Martin. He said that the preliminary experiments have been completed, and it has been shown that the mouse is a good system for infectious DNA by the parenteral route. Infection of hamsters is being added as an assay. The status of provision of P4 containment facilities was also discussed. Pilot experiments may be conducted in a mobile high containment laboratory which is being moved to the NIH.

Dr. Rowe stated that the polyoma experiment is only a minor part of a risk assessment program which should be undertaken. He reported that he and Dr. Martin had already convened a meeting of consultants on

the subject of risk evaluation. He proposed that there should be a screening procedure to try to rule out some of the postulated hazards, and that it is incumbent on the scientific community to produce data. It was the opinion of the consultants that enteric epidemics are extremely remote. Concepts such as this should be discussed in a public forum.

Dr. Rowe stated that as a start at hazard assessment a large number of shotgun clones could be tested for pathogenicity in mouse and rat systems. One could investigate whether certain DNA fragments confer a selective advantage on the bacterial host in such systems. He also suggested the possible use of rabbit ileum loop and guinea pig conjunctiva tests.

The Committee discussed some of the issues associated with biohazard assessment experiments. The point has been raised by critics of the experiments that the hazards are unimaginable. Therefore, the argument is made that tests cannot be designed to assay these kinds of hazards. Concern about transfer of cloned segments to other organisms also needs to be addressed. It was felt that attempts to determine whether bacteria naturally take up DNA of higher organisms should be given high priority. The Committee turned down a motion that grantees who are conducting recombinant DNA experiments be encouraged to determine whether cloned DNA fragments provide a selective advantage to the bacterial host. It was agreed that experiments to assess hazards need to be designed very carefully if they are to provide credible answers. The Committee unanimously recommended that a public symposium on the problem of biohazard assessment should be convened within six months. This symposium might also make recommendations on criteria for EK3 testing. A subcommittee on biohazards, composed of Drs. Rowe, Spizizen, Szybalski and Walters, was appointed.

IX. REVIEW OF CORRESPONDENCE REGARDING GUIDELINES AND RESEARCH PROTOCOLS

The Committee reviewed correspondence requesting clarification of issues related to recombinant DNA technology and the NIH guidelines. The Committee then reviewed research protocols for compliance with the levels of biological and physical containment required by the guidelines. The latter review was conducted in closed session as it dealt with detailed research protocols.

X. DATES OF NEXT MEETING

The Committee will meet on January 15-16, 1977 in Miami, Florida in conjunction with the Miami Winter Symposia.

XI. ADJOURNMENT

The meeting was adjourned at 5 p.m., September 14, 1976.

I hereby certify that, to the best of my knowledge, the foregoing Minutes and attachments are accurate and complete.

Date

DeWitt Stetten, Jr., M.D., Ph.D.
Chairman, Recombinant DNA Molecule
Program Advisory Committee
National Institutes of Health

William J. Gartland, Ph.D.
Executive Secretary

CHAIRMAN

STETTEN, DeWitt, Jr., M.D., Ph.D.
Deputy Director for Science
Office of the Director
National Institutes of Health
Bethesda, Maryland 20014
301 496-1921

VICE CHAIRMAN

JACOBS, Leon, Ph.D.
Associate Director for
Collaborative Research
Office of the Director
National Institutes of Health
Bethesda, Maryland 20014
301 496-3111

ADELBERG, Edward A., Ph.D. (78)
Professor
Department of Human Genetics
School of Medicine
Yale University
New Haven, Connecticut 06510
203 436-0821

CURTISS, ROY, III, Ph.D. (77)
Professor
Department of Microbiology
School of Medicine
University of Alabama
Birmingham, Alabama 35294
205 934-2430

DARNELL, James E., Jr., M.D. (77)
Professor
Department of Molecular Cell Biology
Rockefeller University
New York, New York 10021
212 360-1970

DAY, Peter R., Ph.D. (80)
Chief
Division of Genetics
Connecticut Agricultural
Experiment Station
New Haven, Connecticut 06504
203 787-7421

HELINSKI, Donald R., Ph.D. (78)
Professor
Department of Biology
University of California, San Diego
La Jolla, California 92037
714 452-3638

HOGNESS, David S., Ph.D. (78)
Professor
Department of Biochemistry
Stanford University
Stanford, California 94305
415 497-6166

KUTTER, Elizabeth M., Ph.D. (79)
Member of the Faculty
in Biophysics
The Evergreen State College
Olympia, Washington 98505
206 866-6719

LITTLEFIELD, John W., M.D. (78)
Professor & Chairman
Department of Pediatrics
Children's Medical & Surgical Center
Johns Hopkins Hospital
Baltimore, Maryland 21204
301 955-5000 x5976

REDFORD, Emmette S., Ph.D., LL.D. (79)
Ashbel Smith Professor of
Government and Public Affairs
Lyndon B. Johnson School of
Public Affairs
Univeristy of Texas at Austin
Austin, Texas 78712
512 471-4127

ROWE, Wallace P., M.D. (79)
Chief, Laboratory of Viral Diseases
National Institute of Allergy &
Infectious Diseases
National Institutes of Health
Bethesda, Maryland 20014
301 496-2613

SETLOW, Jane K., Ph.D. (78)
Biologist
Brookhaven National Laboratory
Upton, Long Island, New York 11973
516 345-3420

SPIZIZEN, John, Ph.D. (79)
Member and Chairman
Department of Microbiology
Scripps Clinic & Research Foundation
La Jolla, California 92037
714 459-2390 x402

SZYBALSKI, Waclaw, D.Sc. (77)
Professor of Oncology
McArdle Laboratory
University of Wisconsin
Madison, Wisconsin 53706
608 262-1259

WALTERS, LeRoy, Ph.D. (80)
Director
Center for Bioethics
Kennedy Institute
Georgetown University
Washington, D.C. 20057
202 625-2371

EXECUTIVE SECRETARY

GARTLAND, William J, Jr., Ph.D.
Director
Office of Recombinant DNA Activities
National Institute of General Medical Sciences
National Institutes of Health
Bethesda, Maryland 20014
301 496-2323

SEPTEMBER 1976

RECOMBINANT DNA MOLECULE PROGRAM ADIVSORY COMMITTEE

LIAISON REPRESENTATIVES

HEDRICH, Richard, Ph.D.
Coordination Program of Science
Technology & Human Value
National Endowment for the Humanities
Washington, D.C. 20506
202 382-5996

NIGHTINGALE, ELENA O., Ph.D.
Assembly of Life Sciences
National Academy of Sciences
Washington, D.E. 20418
202 389-6808

LEWIS, Herman W., Ph.D.
Division of Biological and
Medical Sciences
National Science Foundation
Washington, D.C. 20418
202 632-4200

Duda, George, Ph.D.
Division of Biomedical and
Environmental Research
Energy Research and Development
Administration
Washington, D.C. 20545
301 353-5037

A National Recombinant DNA Facility

Earlier this year the Mammalian Cell Lines Committee (MCLC) sent you a letter which suggested the creation of a national recombinant DNA facility. Dr. Stetten has asked me to visit your committee and discuss this concept in greater depth. I shall begin by outlining the historical development of this notion in the context of our committee. The MCLC was officially organized four years ago expressly to oversee the development and day to day operation of a human mutant cell lines repository. It was our responsibility to design a collection of human primary cell cultures which could be representative of the major genetic and cytogenetic human diseases. The purpose of this collection was to provide a set of standard biological materials in the form of cell lines which would be useful in the study of a broad spectrum of human genetic and somatic cell genetic problems. Today, well over a thousand items are stored in the repository, and these are used at an increasing rate by investigators in this country and abroad. Also, I should emphasize the dynamic nature of the collection in the sense that we continue to add to it, and to reorganize and substitute items.

It may be useful to review how the repository works from an administrative point of view. The key to the operation is MCLC. The committee is composed of individuals knowledgeable in the fields of general genetics, human genetics, and somatic cell genetics. They have term appointments

and rotate off the committee in a staggered fashion allowing both change and continuity. The committee is responsible to the NIGMS and makes its reports directly to the NIGMS administration. At the outset, the MCLC was intimately involved in defining the objectives of the cell repository, and then playing an important role in reviewing submitted contract proposals, and finally in the eventual selection of a contractor. Subsequent to letting the contract, the MCLC has reviewed the performance of the repository (Institute for Medical Research, Camden, New Jersey), and has continued to set policy regarding the aims and goals of the overall operation. I believe that whatever success we have enjoyed stems from our administrative design. The following points are critical. The MCLC is independent of the contractor, and because of its revolving nature represents the whole of the scientific community of users. The committee formally advises NIGMS, thus indirectly exercising financial control over the contractor. Power over the purse is essential. We have found these administrative arrangements to be simple and effective. They allow for both continuity and change; they are self-perpetuating, yet self-renewing in the sense of responding to new ideas and needs.

More recently, the role of the MCLC has been broadened. We now serve as a genetics advisory committee to NIGMS. The membership of the committee has likewise been increased so as to reflect an interest in all of genetics. The regulation of the Mutant Cell Lines Repository is now the responsibility of a subcommittee of the enlarged parent committee.

The newly reconstituted genetics advisory committee has been in existence for one year, and that period has been spent largely in defining its new responsibilities. In the coming year, we shall study in some detail questions relating to Genetics Center programs, training programs in genetics, and the issue before us now, the possibility of a national recombinant DNA facility.

The idea of such a facility was put forward by Herbert Boyer about one year ago. The committee members most involved in its conceptualization have been H. Boyer, O. Smithies, and myself. It is accurate to say that the entire committee has reacted favorably to the idea, and believe it should be further explored as a concept. That is to say there has been unanimous support for such a plan at least in principle. The purpose of our meeting today, as I see it, is to make this proposal more specific, more tangible, and attempt to define real problems in terms of its possible implementation.

Firstly, before defining physical aspects of such a facility or its administrative organization, let us focus on its purpose. Discussions this past summer with H. Boyer and others at the Nucleic Acid Gordon Conference indicated that a national recombinant DNA facility would be most useful and effective at least at the outset if limited largely to human DNA. Investigation on human DNA is presently restricted most highly by requirements of high biological and physical containment. Thus, those studies likely to be most beneficial to man are liable to be

the slowest to develop under the present circumstances. It was obvious at the Gordon Conference that experimental systems involving recombinant DNAs of prokaryotes and lower eukaryotes were progressing in a vigorous and healthy way, but that work or human, primate, and mammalian DNAs was advancing less rapidly in spite of the considerable intestest in these systems. We suggest that a properly designed national facility could help alleviate this situation.

Again let us postpone consideration of physical and administrative aspects of implementation and simply imagine what such a facility might do. We would conceive of the facility as a well-equipped, and staffed laboratory which could perform recombinant DNA experimental work at the highest levels of biological and physical containments. The following three major functions would be carried out. (1) The laboratory would itself isolate fragments of the human genome for use by the general scientific community according to an agreed plan. Isolated, cloned DNA fragments could be analyzed outside of the facility by individual investigators under conditions of lower containment, depending on the nature of experimentation. (2) Visiting investigators could bring their experiments to the facility in order to carry out the hazardous biological steps, then return to their home laboratories to perform reduced hazard experiments of a biochemical or cytological nature. And (3) the national facility would serve as a repository, registry, and general clearing house for human DNA fragments.

It is useful to describe in greater detail the cloning experiments which might be performed by the resident staff of such a facility. The following experiments might be contemplated. (1) Random fragments of DNA could be isolated from the various redundancy classes of DNA. At the outset, the number of fragments could be few in number, but increased as required. Such fragments could be used as markers for particular human chromosomes using an in situ hybridization approach, or possibly for individual human chromosomes as isolated in hybrid cells using hybridization kinetics as an analytic procedure. (2) Reverse transcripts could be made from specific mRNAs, or from heterogeneous populations of mRNAs from cell populations of specific epigenetic type. And (3) DNA fragments might be cloned from small defined portions of the human genome isolated by means of newly developing somatic cell genetic procedures. Thus, the facility would serve to produce collections of DNA fragments which investigators could use for certain types of experiments. The data derived from such experimentation would serve to further characterize the DNA fragments in the collection, and thus enhance their usefulness. It should be pointed out that the larger the collection of fragments available for study, the less will be the demand of individual investigators to use the facility for the purpose of isolating their own personal collection of fragments. Even in those instances where visitors do isolate fragments, it should be with the understanding that these materials would ultimately revert to the collection, and thus become accessible to others.

To summarize, we recognize three activities for a national recombinant DNA facility: (1) the production of well-characterized collections of

cloned DNA fragments, (2) a facility which could be used by visiting investigators, and (3) a repository and registry for cloned DNA fragments. What advantages might accrue from the establishment of a national recombinant DNA facility? The following come to mind.

(1) High containment experiments on human DNA would be accelerated.

(2) The number of investigators contributing to human DNA analysis would be increased.

(3) Funds might ultimately be saved by reducing the number of overlapping projects and redundant laboratory facilities.

(4) The early creation of a library of standard materials could impose order and direction on a developing field of study.

(5) The facility would provide experience in the handling of possibly bio-hazardous materials. In this way, the center could serve as a model for other facilities.

(6) The existence of a national facility might reduce anxieties related to the performance of high containment experiments at local levels. City governments might more readily permit experiments at P3 levels of containment, if assured that P4 experiments would be performed elsewhere.

I have left the more complicated issues of organization and implementation until last. In fact, I do not think these matters can be fruitfully discussed here and now in any detail. However, I do believe that the mutant cell bank serves as a useful model. If one were to seriously consider the feasibility of a national recombinant DNA facility, the way to begin is by the constitution of an advisory committee. The advisory committee should be composed of individuals vitally involved in recombinant DNA studies and sympathetic to the concept of a national facility. Such a committee could then advise the NIH regarding the preferred nature of the facility, play a direct role in its implementation, and ultimately regulate its day to day operation.

In closing, I want to emphasize the following two points. One, the Mammalian Cell Lines Committee whom I represent, strongly supports the creation of a national recombinant DNA facility, and its members are prepared to help in planning such as enterprise. Secondly, the Director of NIGMS, Dr. Ruth Kirschstein has asked me to convey to you the Institute's interest in the support of such a venture.

Francis H. Ruddle, Chairman
Mammalian Cell Lines Committee
National Institute of General
Medical Sciences

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

August 27, 1976

As you may know, Stanford University and the University of California have proceeded to file a patent application on a process for forming recombinant DNA. This invention was generated in performance of an NIH grant. A number of other Universities, including the University of Alabama, may also file patent applications on derivatives of recombinant DNA research. Notwithstanding Stanford's right to file under the terms of a prior agreement with the Department, they have solicited NIH's view on an appropriate plan for administration of this invention. A copy of their letter on the matter is enclosed.

These patent activities, the certitude that other important inventions in this field are forthcoming, and the public's apprehension over control of recombinant DNA research compel inquiry into whether the Department's normal policy of allocating invention rights is consonant with the concerns about this research or whether special treatment would be more appropriate.

Invention rights are normally allocated in either of two ways under Department patent regulations:

First, if a University or other nonprofit institution seeks to enhance its technology transfer capability, the Department may enter into an Institutional Patent Agreement (IPA). This provides to the institution the first option to ownership in all inventions made in performance of Department research, subject to a number of conditions deemed necessary to protect the public interest. Some of the more important conditions are

(1) a royalty-free license permitting the Government and those functioning under Government direction to practice the invention,
(2) a limit on the term of any exclusive license granted,
(3) Department authority to withdraw specified grants from the agreement, and
(4) the right of the Department to regain ownership due to public interest considerations or the institution's failure to take effective steps to commercialize the invention.

A more detailed outline of such conditions is enclosed.

Stanford and the University of Alabama each hold one of the 65 IPA's now being administered by the Department.

Second, under grants and contracts with institutions having no identified technology transfer capability, the Department utilizes a provision deferring determination of ownership until an invention has been made. Under the deferred determination provision, an innovating institution may petition the Department for ownership of an invention after it is identified. In the past, approximately 90 percent of all such petitions have been granted on the basis of a satisfactory institution plan for development or licensing, subject, however, to conditions similar to those contained in the Department's IPA's.

The Department's normal policy of allocating invention rights is designed to facilitate the transfer of technology from the bench to the marketplace, by assuring that the innovating institution has the right to convey those intellectual property rights necessary to induce industrial investment and continued development of inventions generated with Department support. Only the IPA policy, however, assures a management focal point in the innovating institution which is trained to solicit and establish timely rights in intellectual property prior to invention.

We have been advised by the Department Patent Branch that 167 patent applications were filed from 1969 through the fall of 1974 under IPA's. Approximately $24 million is committed to the development of inventions on the basis of licenses granted under these patent applications. Meanwhile, we are advised that the Department, under the deferred determination provision, has granted 162 of the institutions' 178 petitions for ownership. Approximately $53 million was invested or committed to development under the licenses awarded. The commitment of private risk capital in these instances is viewed as evidence that a licensable patent right is a primary factor in the successful transfer of Department research results to industry and the marketplace.

It indeed appears that the incentives provided by Department patent policy have encouraged the development of new technology in general and afforded patent protection for some inventions to the economic benefit of the United States.

The control of DNA research envisioned by the guidelines, however, requires a delicate balance between need for rapid exchange of information unhampered by undue concern for patent rights and a potential for achieving uniformity in safety practices through conditions of licensure under patent agreements.

As noted, Stanford has indicated some willingness to consider modification of their IPA as it relates to such research. There are a number of possible policy options, short of the present allocation of rights under the IPA, which could be considered for discussion with Stanford and as possible alternatives to the present allocation of rights made under all other IPA's. Some of these options are as follows:

(1) Institutions could be discouraged from filing patent applications on inventions arising from recombinant DNA research. If this option were pursued, publication would be relied on to cut off all possible adverse patent claims.

(2) Institutions could be asked to file patent applications on inventions arising from recombinant DNA research and to dedicate all issued patents to the public. This would, to a greater extent than (1), block adverse patent claims.

(3) Institutions could be asked to assign all inventions made in performance of recombinant DNA research to the Department. The Department as assignee of the invention could either pursue the licensing of whatever patent applications were filed or dedicate issued patents to the public.

(4) The Department could continue to permit institutions to exercise their first option to ownership under the IPA but require that all licensing of patented inventions be approved by the Department. The Department could set certain conditions for approval, such as compliance with the NIH guidelines on recombinant DNA research.

(5) The Department could permit institutions to retain their first option as in (4), but approve only exclusive licenses. Here, as above, the Department could set out conditions to account for the special nature of recombinant DNA research, both in approved exclusive and non-exclusive licenses.

If it is determined that institutions with IPA's should be permitted to retain ownership of inventions arising from recombinant DNA research, I am concerned about the effect of the processing of patent applications on the dissemination of research information. Under United States law, an inventor has a one-year period of grace after research results are published in which to file in order to obtain a valid United States patent. However, valid protection in a number of foreign countries requires that a patent application be filed prior to publication. If one publishes first, valid patent protection cannot be obtained in such countries. Our patent people believe that any necessary patent applications can be handled expeditiously without an undue burden on disclosure. I am especially mindful

of your Committee's concern for the rapid dissemination of research results in recombinant DNA research and would especially welcome your thoughts on this matter. For example, would you view patent claims as an impediment to the operation and functions of your Committee? What experience, if any, have you or your colleagues or institution had with patent claims in this regard?

As you know, about an hour of the meeting's agenda will be devoted to your review of patent policy, and I have asked Joe Perpich and Norm Latker, the Department Patent Counsel, to attend the meeting for this discussion.

I would appreciate your views on Department patent policy as it relates to the conduct of your research, the operations of your Committee, and the suggested policy options I have outlined above. I intend also to solicit advice on this matter from other interested parties in the scientific community and in the public and private sectors.

Thank you very much for your consideration of this most important matter.

Sincerely yours,

Donald S. Fredrickson, M.D.
Director

3 Enclosures

Yale University New Haven, Connecticut 06510

Attachment IV

SCHOOL OF MEDICINE
333 Cedar Street
Department of Human Genetics

To: Dr. DeWitt Stetten, Jr.
Deputy Director for Science
National Institutes of Health
Bethesda, Maryland 20014

From: Dr. Edward Adelberg, Chairman, Working Group on Safer Hosts and Vectors

The Working Group on Safer Hosts and Vectors met on June 9, 1976, to review the data submitted to us concerning several proposed EK2 host-vector systems. The original group, consisting of Drs. Davis, Falkow, Spizizen, Stocker, Szybalski, Thomas, and myself, was augmented by the appointment of Drs. Susan Gottesman, Nancy Kleckner and David Dressler in order to provide additional expertise on host-vector systems in which the vectors are lambda phages.

In order to insure that all submitted data receive full consideration, we split up into two sub-groups. One group devoted its entire attention to host-plasmid data, the other to host-phage data. The groups were:

Group 1 (Host-plasmids)	Group 2 (Host-phages)
Dr. E. Adelberg (Chairman)	Dr. C. Thomas (Chairman)
Dr. B. Davis	Dr. D. Dressler
Dr. S. Falkow	Dr. S. Gottesman
Dr. J. Spizizen	Dr. N. Kleckner
Dr. B. Stocker	Dr. W. Szybalski

Group 1 met from 7:30 P.M. to 11:00 P.M. in the Bush Room of M.I.T.'s Building 10 in Cambridge, Mass., on June 9, 1976. Group 2 met in the nearby Room 155 of Building 4 from 7:30 P.M. to 12:30 A.M. the next morning. Drs. Roy Curtiss and Don Helinski attended the meeting of Group 1 in order to answer questions concerning their data; Dr. Fred Blattner was present at the meeting of Group 2 for the same purpose. Both meetings were open to the public; Group 1's meeting was attended by about fifty observers, and Group 2's by about ten. Dr. Leon Jacobs worked with Group 1, and Dr. William Gartland worked with Group 2.

In this letter, I will report to you on the analysis by Group 1 of two host-plasmid systems, χ1776 (pSC101) and χ1776 (pCR1). The analysis by Group 2 of several host-phage systems will be reported to you separately by Dr. Charles Thomas.

I will start by saying that at the conclusion of the meeting, Group 1 voted unanimously (5-0) to report that in the opinion of its members both of the above-listed host-plasmid systems* meet the criteria for EK2 as stated in the N.I.H. Guidelines. In order to reach this conclusion, we found it necessary to interpret the Guidelines with respect to the following questions: (1) Under what specific conditions, and within what time period, must the data indicate a 10^{-8} or lower probability of survival of the host? (2) Under conditions in which survival is prolonged, such as in tap water, can the criteria be satisfied by the demonstration of a 10^{-8} or lower probability of plasmid transmission? Our answers to these questions will become apparent as you read the following summaries of our considerations.

The Guidelines state (p. 25) "For EK2 host-vector systems in which the vector is a plasmid, no more than one in 10^8 host cells should be able to perpetuate the vector and/or a cloned DNA fragment under nonpermissive conditions designed to represent the natural environment either by survival of the original host or as a consequence of transmission of the vector and/or a cloned DNA fragment by transformation, transduction or conjugation to a host with properties common to those in the natural environment." We will discuss the data pertaining to survival and transmission separately, in the following sections.

I. χ1776 (pSC101)

Data on this system were provided by Dr. Roy Curtiss III. We paid particular attention to data obtained with χ1876, which consists of χ1776 carrying the pSC101 plasmid. Although the plasmid in χ1876 contains no inserted foreign DNA, we consider it to be the best available model of the host-plasmid system that will be used by investigators to clone inserted fragments. Many of the data obtained with uninfected χ1776 cells were also found to be extremely useful in predicting the behaviour of host-vector systems based on this bacterial strain.

---

* In a telephone poll taken after the meeting, the committee agreed that the application of this statement to χ1776 (pCR1) should be conditional on the demonstration that thymine-requirement and bile-salt sensitivity are retained.

A. Survival:

For the purposes of our considerations, we defined the natural environment of E. coli as the intestinal tract of man or other warm-blooded vertebrates*. Since the average time of passage (from ingestion to excretion) in man is about 24 hours, we considered this to be a reasonable time limit within which the 10^{-8} survival level should be reached. This rate of host cell death would effectively prevent any cells from reappearing in a permissive environment, as well as preventing them from expressing harmful gene products or transmitting the vector during their residency in the gut.

(1) Survival in growth media: Experiments with χ1876 in a variety of laboratory media show that the host cells undergo rapid death under conditions optimal for growth of normal strains as a result of three properties: requirement for diaminopimelic acid (DAP), coupled with the inability to synthesize colanic acid; requirement for thymine (T); and extreme sensitivity to bile salts and other detergents. In a few of these experiments the 10^{-8} level of survival was reached in 24 hours or less; in others, longer periods were required, or the experiment was terminated before the 10^{-8} level was reached. In one experiment, re-growth occurred after six logs of death; this was presumably due to the large amount of DAP liberated by cell lysis. Other experiments suggest that such regrowth would not occur in the natural environment for two reasons: (i) the DAP cells could not conceivably reach the lower bowel at such high cell densities, so that the concentration of liberated DAP would be negligible, and (ii) any free DAP would be rapidly competed for by the normal flora.

In any case, we do not consider that the media and conditions used represent the "natural environment," and we therefore do not consider it mandatory that the limits set by the Guidelines for the extent and rate of death be met in all such experiments. Rather, we consider the main purpose of these experiments to be the demonstration of those properties of χ1776 which underly its candidacy for an EK2 host.

(2) Survival in non-growth media: The data on survival of χ1776 and χ1876 indicate that these strains are generally no more sensitive to incubation in tap water or to dessication than are ordinary strains of E. coli K12. Thus, in eight days χ1876 underwent about three logs of death in tap water and about five logs in the dry state. As discussed below, however, we consider the inability of χ1876 to transmit its plasmid under these conditions to compensate for its low rate of death, so that the criteria of eight logs of death within 24 hours are not applicable here.

---

* We based this interpretation on the view, generally held by public health bacteriologists, that the presence of typical E. coli in water is a reliable indication of its recent contamination by fecal matter from mammals or birds, and — by inference — that typical E. coli strains, though they can survive for variable periods in water or soil, do not persist there indefinitely, as they would do if able to multiply in such environments.

(3) Survival in the intestinal tract of the rat: The conditions used in these experiments do indeed represent the natural environment as we have defined it. The data of Curtiss's Table 17 show that the survival of χ1876 falls well below 10^{-8} within six hours, as measured in feces following feeding by stomach tube.

Curtiss measured survival of χ1876 itself, rather than survival of a marker on the vector as required by the Guidelines. Our conclusion that χ1776 (pSC101) meets the criteria for an EK2 system is thus not based on a direct test of vector-marker survival, but rather on data given separately for host cell survival and the probability of vector transmission.

B. Transmission

In order for χ1876 to transmit its plasmid, three events must take place: (1) χ1876 must come into contact with a donor of a conjugative, mobilizing plasmid; (2) it must receive this plasmid by conjugation, transduction, or transformation; and (3) it must transfer pSC101 by mobilization to a recipient bacterium.

The probability of all three events occurring is the product of their separate probabilities; given the frequency in nature of derepressed, conjugative plasmids, and the cell densities which may be expected in non-growth environments, the first two events have a very low probability of taking place. Multiplying this probability by the rate of transmission by χ1776 of even a derepressed plasmid (R1drd19) in tap water ($<2 \times 10^{-8}$, see Table 52) we conclude that transmission in non-growth environments has a negligible probability.

In growth media, the probability of the first two events is obviously higher. The ability of χ1776 to transmit a plasmid under such conditions, however, declines in parallel with survival (Table 23).

C. Conclusions

A cell of strain χ1776 (pSC101) which escapes the laboratory may encounter a set of conditions ranging from those which are totally incapable of supporting growth (e.g., tap water) to those which permit growth of normal E. coli cells (e.g., water containing available nutrients, or sewage). At the one extreme, χ1776 (pSC101) is rendered safe by virtue of the negligible chance that it will transmit its plasmid; at the other extreme, it is rendered safe by virtue of its rapid and extensive death. Under intermediate growth conditions, we infer that the two opposing tendencies will continue to cancel each other out: the greater the chance of survival, the lower the chance of transmission, and vice-versa. These considerations, together with the demonstration that in the rat intestine the 10^{-8} survival level is reached in less than six hours, lead us to conclude that χ1776 (pSC101) meets the criteria for an EK2 system.

χ1776 (pCR1)

Dr. Helinski has submitted data showing that this strain achieves eight logs of death in 24 to 72 hours, in a laboratory medium lacking DAP. In some

these experiments a second E. coli strain was present; the data show that the plasmid was not transferred to cells of the second strain.

Under these conditions, at least, substituting the plasmid pCR1 for pSC101 does not alter the basic property of DAP-less death of the host cells. We have therefore tentatively concluded that χ1776 (pCR1) meets the criteria for EK2. This conclusion is tentative because it rests on the assumption that substituting pCR1 for pSC101 does not alter the host cell's sensitivity to bile salts or its requirement for thymine — properties that are critical — to the rapid death of the cells under conditions representing the natural environment.

III. Recommendations concerning the use of χ1776 (pSC101) and χ1776 (pCR1)

The slow growth of these strains makes the chance of overgrowth of their cultures by other strains a serious problem. Such overgrowth might represent faster-growing variants (e.g., as might arise from reversion of characters not determined by deletions or multiple mutations), or faster-growing contaminants of the cultures. We therefore recommend (1) that users be urged to add cycloserine to all cultures of these strains (which are cycloserine-resistant), and (2) that users be reminded of their responsibility to check clones carrying foreign DNA to ensure that they retain the characteristics of DAP-less death, thymine-less death, and bile salt sensitivity.

IV. Recommendations for future EK2 strains

Although the strains discussed here appear to us to meet EK2 criteria, they lack a number of desirable features that new EK2 strains constructed in the future might incorporate. Two suggestions are:

(1) Mutations should be introduced in the plasmid to make it dependent on a particular host (e.g., one carrying certain suppressor mutations), thus reducing further its chance of escape.

(2) The strains should grow rapidly under permissive conditions, thus reducing the chance of overgrowth by revertants or contaminants.

The desirability of these and other features were pointed out to us in written critiques submitted by Drs. Adhya and Enquist of N.I.H. and by the Boston Area Recombinant DNA Group. We found most of their points well taken, and considered them as best we could (given the circumstance that we received them only a day or two before our meeting). In one or two instances we disagreed with their conclusions, however. For example, the authors of the critiques deduced from Curtiss's data that the presence of pSC101 increases the survival of χ1776; the experiment which appeared to show this, however, was not reproducible. Also, the critiques suggest that the property of minicell production exhibited by χ1776 might pose a hazard; we think not, given the fact that minicells are unable to transmit plasmid DNA to other cells.

Summary

The host-vector system, χ1776 (pSC101), appears to us to meet the criteria for EK2, as we interpret the definition of EK2 in the Guidelines. The host-

vector system, χ1776 (pCR1), will meet these criteria as soon as data are submitted to show that substituting pCR1 for pSC101 does not alter the thymine requirement or the bile salt sensitivity of the host. We understand that Dr. Helinski plans to submit such data in the near future. The assumptions which we made in reaching these conclusions are explained. Recommendations for the use of these strains in the cloning of foreign DNA, as well as for the construction of future EK2 systems, are presented.

REPORT TO THE RECOMBINANT ADVISORY COMMITTEE[1/]

FROM THE WORKING GROUP ON SAFER HOSTS AND VECTORS: LAMBDA PHAGE SYSTEMS

The meeting of September 12, 1976, was convened at 12 noon in Conference Room 9, Building 31C, of the NIH. Items on the Agenda were as follows:

I. Design of "standardized" laboratory tests for EK2 certification.

II. Consideration of two applications for EK2 certification of λ phage vectors.

III. Modifications in the definition of EK2 phage vectors.

IV. Other business.

I. Laboratory Tests

Based, in part, on the written suggestions of various members of the Working Group, four criteria were proposed to embody the essential requirements for certification. These criteria were approved unanimously by the Working Group to be recommended for adoption by the Advisory Committee in certification of λ EK2 vector systems.

A. Yield of Vector Phage

The yield of vector phage (+ or - a model cloned fragment) propagated under laboratory conditions should be no less than 10^{10}/ml of unconcentrated crude lysate.

1/ This report was revised and corrected at a subsequent meeting of the Working Group on December 13, 1976.

B. The probability that a fragment cloned on the vector will form a persistent association with the permissive host used for its propagation and that this complex will then survive for 24 hours outside the laboratory should be less than 10^{-8}. This probability is calculated as the product of (1) and (2) below.

(1) Number of fragment-containing surviving bacteria per output fragment-containing phage in the lysate.

(2) Survival of bacteria after 24 hours under non-permissive test conditions as determined in a separate experiment carried out in the absence of phage.

Comments on:

(1) This fraction should be measured both at the time of lysis and at 24 hours after lysis with the culture maintained under optimal growing conditions. The denominator in both cases with be the titer of phage at the time of lysis. The number of fragment-containing bacteria should be measured in an appropriate manner with justification by reconstitution experiments.

As an example, such a test might measure the number of gal^+ bacteria formed after infection of a permissive host (lacking the gal base sequence homology with the cloned fragment) with a vector containing a gal^+ (model cloned DNA) fragment. Demonstration that authentic gal^+ colonies could be detected at the frequency measured in the test would constitute appropriate justification.

(2) The Working Group does not wish to specify at this time non-permissive conditions, that must be met in this test for all cases.

The two tests suggested in current applications: "survival in raw sewage" and "survival in tap water," though useful, refer to survival in nature and are therefore more appropriately considered in the context of EK3 certification.

For the present, the safety factor characteristic of any particular host can be considered independently for each application.

An example of the application of this principle is the host for a current λ EK2 candidate, which in case of culture overgrowth for 24 hours may reach a level of persistent association of about 10^{-6} associations per fragment-containing vector. Thus, production of fragments with these vectors should be limited to hosts offering a factor of safety of at least 10^{-2}. A strain related to χ1776, whose survival in the rat intestinal tract 24 hours after feeding can reasonably be estimated to be about 10^{-4}, was therefore accepted by the Working Group for use with the two proposed EK2 systems.

A minimal test for any proposed modified host, however, will include a measure of the reversion frequencies for each of the relevant mutations.

Although killing by chloroform decreases the probability of survival of any clone-containing bacteria by many orders of magnitude, this factor

should not be included in calculating the degree of biological containment measured in this test.

C. The frequency of survival of fragment-transferring capacity of a vector phage carrying a model fragment should be less than 10^{-8} in the tests specified below.

It is understood that this value (as in B) should represent the product of the following probabilities:

(1) The probability that the phage will survive until it meets a sensitive host.

(2) The probability that (a) the fragment will persist in a non-permissive λ-sensitive host or (b) that the fragment-containing vector phage will acquire genetic material from a homologous prophage that will serve to negate the safety features of the vector.

Comments:

Data available for wild-type λ suggests that (1) is less than 10^{-3}. Theoretically then, the value for (2) need be no less than 10^{-5}. Nevertheless, the Working Group considers it essential that there be some estimation of the "worst possible case" relative to this parameter. We can very conservatively estimate that this case will actually arise in less than one of ten (10^{-1}) λ-sensitive strains encountered in nature. We therefore recommend that the following "worst-case" tests be included and show:

(a) The number of fragment-containing bacteria per adsorbed input fragment-containing phage should be less than 10^{-4} and

(b) The number of fragment-containing rearmed (wild-type) phage per adsorbed input fragment-containing phage should be less than 10^{-3} for genetic safety features located to the left of the cloned fragments and less than 10^{-3} for similar features located to the right.

It is understood that the host in this case should be a non-permissive lysogen bearing a heteroimmune prophage which has base-sequence homology with the input phage and a compatible late gene regulatory system.

D. The product of reversion frequencies of "disarming" mutations should be less than 10^{-8}.

Comment:

This test should be done in non-permissive, non-lysogenic bacteria. Separate determination of the reversion frequencies of individual mutations should be made wherever possible and the product of these should be less than 10^{-8}. Restriction-modification barriers should not be included in this test.

II. Two applications were considered. Although it was recognized that the data provided were accumulated before the specific tests outlined in I were available, they were discussed and analyzed in the context of these new parameters.

Blattner et al. Application

Re: Vectors Charon 3A and 4A

(a) With respect to the phages themselves, data were presented or provided which could reasonably be considered to meet the tests specified in I, with one exception relating to Item C(2)b, i.e., rearming of the fragment-containing vector by acquisition of genetic material from the prophage of a non-permissive lysogen. Since in this case two types of "disarmament" are employed (with the loss of either rendering the vector "unsafe") each must be independently tested and each must pass the $\pm 10^{-4}$ safety level.

Specifically after infection of the *E. coli* Δ*lac* (Aλ*B*λh80*att*80*imm*21QSR80) host the number of *lac*$^+$-containing amber$^+$ phage per input *lac*$^+$ Charon phage should be less than 10^{-4}. The assays should be performed after the time of lysis normally observed for wild-type λ and without addition of chloroform.

(b) With respect to Item B(2) it is the estimation of the Working Group that the host, DP50, provides a safety factor equivalent to at least 10^{-4} when used for propagation of the vector which carries a cloned fragment. Thus, the requirement stipulated in Item B, even in conditions of

overgrowth (test B(1) after 24 hours) where a safety factor of 10^{-6} was obtained, are satisfied when this host is used for propagation.

Re: Vectors Charon 316A, 414A

Data for tests analogous to Item C are not available.

Re: Vectors Charon 315A, 413A

Data for tests analogous to Items B(1) and C are not available.

Recommendation

The Working Group recommends: (1) That Charon 3A and 4A be certified subject to receipt and approval of data specified for test of Item C(2)b, as outlined above. This approval may be obtained by ballot of the subcommittee through the mail; (2) That Charon 316A and 414A be certified subject to receipt and approval of data specified in Item C, with approval by ballot through the mail; (3) That Charon 315A and 413A not be certified at this time because of insufficient data.

Leder et al. Application - λWES.λB

Data were presented which were considered to meet the criteria outlined for A and D. Indicative data for B and C have been presented but complete data for B(1) and C are required.

Specifically for B(1): After infection of the permissive host by the model gal$^+$ vector phage, the product (1) x (2) should be less than 10^{-8} where

(1) = number of gal$^+$ bacteria per output gal$^+$ phage.

(2) = surviving fraction of host after 24 hours.

A DP50supF host derived from that used by Blattner et al. may be assigned the same 10^{-4} safety factor allowance in B(2) as DP50 itself. No safety factor for large-scale phage propagation is assigned to the 803-8 strain they describe.

The data submitted for the 803-8 strain justify its use in transfection experiments leading to the formation of single plaque clones.

Specifically for C(2)a:

The host should be a supO galΔ and lysogenic for the heteroimmune lambdoid phage with base sequences derived from λ.

Infection should be made at 30°C and at 37°C.

Specifically for C(2)b:

The host should be a supO lambdoid lysogen.

The number of gal^+ W^+E^+ phage per input gal-vector should be less than 10^{-4}.

The number of gal^+ S^+ phage per input gal-vector should be less than 10^{-4}.

Recommendation

The Working Group recommends that λWES.λB be approved for certification subject to receipt and approval of data specified above for tests of B

and C(2)a and C(2)b. This approval may be by ballot by the subcommittee through the mail.

III. Modifications in the definition of EK2 phage vectors.

The Working Group recommends specifically that: Lysogens of any EK2 vector carrying a cloned fragment formed in vitro should be considered as EK1 systems.